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Dear Ken,

It was good to hear from you and about your attempts to get at nucleotide sequence determination of DNA. Although we put in a fair amount of development work in trying to utilize the ribonucleotide trick for study of nucleotide sequences in DNA, we have never pushed it further and I do not forsee that we will get back to it in the near future.

Unfortunately, this summer is almost the very worst time you could have suggested. First of all, during much of June I will be away (Cold Spring Harbor Meeting and Gordon Conference) or involved in organizing this year's Gordon Conference (June 20 - 24). On about July 1 I will be going to Greece for about three weeks to participate in a course in Molecular Biology. I probably will be back by late July and will spend August and September in Palo Alto. However, it's very likely that our entire department will be in a turmoil because of construction. As a result of our gaining new space, considerable renovations are being made and we shall probably have to double up for part of the time. I fear that the summer is apt to be quite hectic and not very conducive to work. This, of course, is not a very inviting picture for you, but I don't think I can be too much more encouraging.

Let me tell you however of some of my impressions about the utility of using the ribonucleotide trick for DNA nucleotide sequence determination. In principle the method should work well and certain of the practical aspects worked out even better than I expected. The major difficulty is in knowing what the DNA polymerase is copying since what one looks at is the <u>product</u> of the enzyme reaction and not the native DNA <u>per se</u>. If only selected regions are copied under these conditions, the measured distribution of different sequences would be biased. The trouble is that there is no good standard small (less than 100 nucleotides) DNA with which to check the method; we tried that with the cyt b2 (BBA <u>108</u>, 243, 1965) but that wasn't the answer.

In any case, the experiment I did to test the methodology was as follows. I used calf thymus DNA as primer and dATP(C^{14}), dTTP(C^{14}), dCTP(C^{14}) and rCTP-α-P^{32} as substrates in the presence of Mn^{++}. The product was recovered by acid-precipitation, digested with alkali and after adding the markers Cp, ApCp, GpCp and ApAoCp the mixture was chromatographed on a Tener Column (7 M urea and a salt gradient). A succession of peaks were eluted; the first contained only Cp^{32} (no C^{14}) and the next two peaks coincided with the dinucleotide and trinucleotide markers. As expected, there was an increasing ratio of C^{14}/P^{32} the later the fractions were eluted. Each of the first four peaks were pooled, urea was removed and the contents subjected to two-dimensional mapping a'la Rushizky. The maps were autoradiographed with X-ray film and the films showed that peak 1 contained only Cp^{32}, peak 2 contained three radioactive spots, two of which nearly coincided with the ApCp and GpCp markers and the third appeared in a region consistent with TpCp. Peak 3 gave 5 clearly discernible radioactive spots (there was also one other very faint one) one of which was very close to the ApApCp marker. The three dinucleotides (these are the only ones possible if the hypothesis upon which the method is based is correct) were eluted, degraded in different ways and shown to be what I've mentioned above. The 5 trinucleotide spots were eluted and rechromatographed in 30% saturated ammonium sulfate. Two of them gave only a single spot and were shown to be ApApCp and TpTpCp, respectively. One gave two spots in about equal amounts and these turned out to be TpApCp and ApTpCp. The other two each gave two spots and in each instance one of the two spots contained approximately 80 to 90% of the label. The predominant trinucleotides were GpApCp and GpTpCp and we believe the minor components were the isomers ApGpCp and TpGpCp, respectively, but the latter two were not thoroughly characterized. The one very faintly visible trinucleotide spot was never identified but may have been GpGpCp.

Actually, it was a mistake for us to have used calf thymus DNA to practice on because it was already known from Josse et al. that the sequence GpC was very low in this DNA and one would predict that the frequency of GGC, TGC and AGC would be very low. I should point out that C^{14}-labeled deoxynucleoside triphosphates were used in this experiment to facilitate the task of proving the composition and sequence of the trinucleotides. The dinucleotides are easily analyzed because they have the form Xp^*Cp^* and on spleen phosphodiesterase they yield Xp^* and Cp^* which are readily identified. But with a trinucleotide $XpYp^*Cp^*$ there would be no way of identifying the base in position X.

The major problem at present is, as I mentioned above, the lack of assurance of the fidelity of copying of double-stranded DNA by the DNA polymerase. So far, from Kornberg's experience, I would say the best candidate is M13 single-stranded DNA as primer and converting it to a completely

double-stranded circle (the newly synthesized strand is believed to be open). In this system no branched DNA is made until after one replication. One could by using the ribonucleotide trick here obtain the family of fragments from the complement to the M13 phage DNA. I don't know, however, how well M13 is copied with Mn^{++} and a ribotriphosphate. We also had talked at one time of looking at the terminal sequence of λ DNA. Quite possibly by copying the terminal regions to produce fully double-stranded molecules (Strack and Kaiser) with a ribonucleotide only in the newly copied portion, one might derive fragments from that short sequence.

One other difficulty which we also were not able to overcome and which limits the method to some extent is the inability to substitute a U or r U for the dT residue. For some reason UTP or rTTP is not significantly incorporated under conditions where the other ribonucleotides work.

I'm sorry that I can't be more encouraging about your wish to come here this summer, but it probably would not be the most conducive place for what you want to do in any case. We could certainly send you some of the enzyme and most of the P^{32} ribonucleoside triphosphates are available commercially. If I can be of any help I'd be glad to try.

With best regards,

Sincerely,

Paul Berg

PB:cm